Animal Health Prod and Hyg (2025) 14 (1) : 10 - 16

Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Investigation of the Gastroprotective Activity of Propolis Extracted in Different Solvents

Ali SORUCU[1*], Osman BULUT[2], Ayşe Nur AKKOÇ[3], Asya Su TUNCER[4]

[1*]Mugla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine, Department of Pharmacology and Toxicology, Muğla, TÜRKİYE
[2]Mugla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine, Department of Surgery, Muğla, TÜRKİYE
[3]Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Department of Pathology, Aydın, TÜRKİYE
[4]Mugla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine, Muğla, TÜRKİYE

ABSTRACT

Propolis is a product produced by honeybees that collects the resins of plants. Secondary metabolites, including phenolic compounds, which are found in the resin of plants and are incorporated into propolis, give propolis many biological properties. Propolis exhibits various properties such as antibacterial, antiviral, antifungal, antioxidant, immunomodulator, anti-inflammatory, antiulcer and wound healing accelerator. The properties vary depending on the source of the plant, season, altitude, climate zone and extraction solvent. The present study investigated and compared the effects of different propolis extracts prepared using alcohol, water, dimethyl sulfoxide (DMSO), and olive oil on the indomethacin-induced gastric ulcers in nine group of Balb/C mice (n=8). Epithelial loss, erosion, bleeding, edema, inflammatory cell infiltration, and mean clinical scores results were evaluated statistically between different propolis extracts compared to omeprazole. Since the histopathological results were remarkably similar, the general assessment was made with the mean clinical score. It was found that DMSO and olive oil extracts of propolis had gastroprotective effects similar to omeprazole. In contrast, hydro-alcohol and water extracts did not show significant differences compared to their solvents and gastroprotective activity. In conclusion, it was determined that the olive oil extract of propolis, which is especially suitable for direct consumption, has the potential to be used as a gastroprotective.

Keywords: *Apitherapy, honeybee, gastroprotective, DMSO, olive oil, propolis*

Farklı Çözücülerde Ekstrakte Edilen Propolisin Mide Koruyucu Etkinliğinin Araştırılması

ÖZET

Propolis bal arılarının bitkilerin reçinelerini toplayarak oluşturdukları bir üründür. Bitkilerin reçinesinde bulunan ve propolise geçen içerisinde fenolik bileşenlerinde bulunduğu sekonder metabolitler propolise birçok biyolojik özellik kazandırır. Propolisin antibakteriyel, antiviral, antifungal, antioksidan, immunmodülatör, antiinflamatuvar, antiülser ve yara iyileşmesini hızlandırıcı gibi birçok özelliği bulunmaktadır. Bu özellikler çevredeki bitki örtüsü, mevsim, yükseklik, iklim kuşağı ve ekstraksiyon çözücüsüne göre değişkenlik göstermektedir. Bu çalışmada, hidro-alkolik, su, dimetilsülfoksit (DMSO) ve zeytinyağı kullanılarak hazırlanan farklı propolis ekstraktlarının her grupta 8 adet Balb/C fare olacak şekilde 9 grupta indometazin ile indüklenen gastrik ülser üzerindeki etkileri araştırılmış ve karşılaştırılmıştır. Epitel kaybı, erozyon, kanama, ödem, inflamatuar hücre infiltrasyonu ve ortalama klinik skor sonuçları, omeprazole kıyasla farklı propolis ekstraktları arasında istatistiksel olarak değerlendirildi. Histopatolojik sonuçlar birbiri ile benzer olduğu için genel değerlendirme ortalama klinik skoru ile yapılmıştır. DMSO ve alkol ekstrakt propolislerin omeprazole benzer gastroprotektif etkilerinin olduğu hidro-alkolik ve su ekstraktının ise kendi çözücülerine göre önemli fark oluşturmadığı ve gastroprotektif etkinlik göstermediği belirlenmiştir. Sonuç olarak, özellikle doğrudan tüketime uygun olan propolisin zeytinyağı ekstraktının gastroprotektif olarak kullanılabilme potansiyeli olduğu belirlenmiştir.

Anahtar kelimeler: *Apiterapi, bal arısı, mide koruyucu, DMSO, zeytinyağı, propolis*

***Corresponding author:** Ali SORUCU, Mugla Sıtkı Kocman University, Faculty of Milas Veterinary Medicine, Department of Pharmacology and Toxicology, Muğla, TÜRKİYE. alisorucu@mu.edu.tr*

Received Date: 24.01.2025 - Accepted Date: 27.03.2025. DOI: 10.53913/aduveterinary.1626085

Introduction

Gastric ulcer is one of the most common diseases of the upper digestive system (Ramakrishnan and Salinas, 2007; Abumunaser, 2021; Ruiz-Hurtado et al., 2021a). While ulcerative lesions in the stomach are primarily observed in the curvature part, ulcerative lesions can also be observed in all gastric tissues from the pylorus to the cardia (Malfertheiner et al., 2009). Among the etiological factors of peptic ulcer, endocrine disorders, acute and chronic renal damage, and various neoplasms of gastric origin have been reported (Kuna et al., 2019). Apart from these etiological factors, the most common factor causing gastric ulcers is the use of non-steroidal and steroid-derived drugs. The pathophysiology of gastric ulcer is caused by disorders of mucosal protective mechanisms such as mucus, bicarbonate, prostaglandin (PG) synthesis in the stomach and duodenum, microcirculatory problems and disruption of the acid-pepsin balance, which can damage the mucosa. As a result of these factors, the gastric mucosal barrier is weakened, and acid release is increased, resulting in damage to the gastric epithelium (Ramakrishnan and Salinas, 2007; Abumunaser, 2021; Ruiz-Hurtado et al., 2021a). Propolis has been revealed to have many biological activities such as antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory and antiulcer (Stojanović et al., 2020). Various studies have also found that propolis has effects such as accelerating the osteogenic process and increasing regeneration in various tissues such as bone, cartilage and dental pulp (Ekeuku and Chin, 2021). In addition, propolis is also known to have a protective effect against gastric ulcers, one of the most common diseases of the digestive system (de Barros et al., 2007; Abd El-Hady et al., 2013; Ruiz-Hurtado et al., 2021a).

Many methods are used to treat peptic ulcers. However, these methods can cause adverse effects on the patient (Ruiz-Hurtado et al., 2021a). Therefore, many natural preventive and therapeutic alternatives are used. Propolis, used in traditional and complementary medicine, is one of the most important natural products used for this purpose. Propolis is a resinous substance, sticky, with a distinctive odour and varying in colour from light to dark brown, produced by worker bees when they bring nectar collected from growing parts of trees such as leaves, buds, branches and shoots to the hive, where it undergoes biochemical changes with wax and various enzymes they secrete. Propolis is an important bee product that has been used in traditional medicine since ancient times to treat many diseases (Sorucu, 2019; Stojanović et al., 2020).

The effect of propolis is due to the active substances such as phenolic compounds in propolis. Many factors affect the presence of these active substances in propolis (Stojanović et al., 2020). In addition, the extraction method and solvent selection affecting the presence of these phenolic substances in the final product are fundamental (Kekeçoğlu and Sorucu, 2021). While ethanol is the most preferred solvent, solvents such as water, methanol, methylene chloride, dichloromethane, lactic acid, hexane, ethyl acetate, acetone, olive oil, β-cyclodextrin, dimethyl sulfoxide, propylene glycol, ethyl acetate and chloroform are also frequently used in various studies. The different solvents used significantly affect the pharmacological properties of propolis since they cause a chemical alteration in the soluble active compounds (Oruç et al., 2023).

Although the gastroprotective activity of propolis has been demonstrated, only a study has been found to investigate the gastroprotective effect of propolis extracted in different solvents against gastric ulcers (Ruiz-Hurtado et al., 2021a; Sahin et al., 2023). The study compared the effects of ethanol and water extract propolis (Sahin et al., 2023). The present study aimed to investigate and compare the protective effects of propolis extracted with four different solvents (water, alcohol, DMSO, olive oil) against indomethacin-induced gastric ulcers in mice models.

Material and Methods

Propolis Extraction

The raw red propolis used in the study was purchased from Muğla (Apitonic-Bee Happy Beekeeping). The propolis was homogenised by grinding it into powder with a grinder. 150 g of propolis was weighed for each extraction, and 450 ml of each extraction solvent (ultrapure water, cold-pressed olive oil, 50% DMSO and 70-30% ethanol-water) was added. The mixtures were shaken in an orbital shaker for one week and filtered through Whatman No1 filter paper to obtain extracts (Kekeçoğlu and Sorucu, 2021; Sorucu and Oruç, 2019). Propolis was taken 1 ml into tared tubes, solvents were evaporated, and resin ratios were determined. The extracts were stored at +4 C until the experimental work.

Animals and Experimental Design

The study was conducted with the approval of Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee under approval number 2022/04 (Date of approval 30/05/2022). A total of 72 male BALB/c mice with a live weight of 15-25 g were used in the study. The mice were provided by the Experimental Animal Application and Research Centre of Muğla Sıtkı Koçman University. The experimental study was carried out in this centre under conditions suitable for mice.

Mice were randomly divided into nine groups of eight animals each. The propolis extracts were applied at 100 mg resin/kg. (Ruiz-Hurtado et al., 2021b).

Since the ulcer-causing potential of indomethacin is higher than other NSAIDs, the preparation was chosen to create an ulcer model. The groups were first treated with propolis extracts and one hour later given indomethacin at 100 mg/kg to induce ulceration (de Barros et al., 2007).

The groups were formed, and treatments were administered via oral gavage as follows.

- Group 1 (OMP): Omeprazole was administered at 30 mg/kg as a positive control.

- Group 2 (WEP): Propolis dissolved in ultrapure water was administered (100 mg/kg).
- Group 3 (W): Ultrapure water (ELGA) 1 ml was administered as a negative control (equal volume WEP).
- Group 4 (EWEP): Ethanol (Merck)-water (70%-30%) extract of propolis was administered (100 mg/kg).
- Group 5 (EW): Ethanol-water (70%-30%) was administered as a negative control (equal volume EWEP).
- Group 6 (DMSOEP): DMSO (Tekkim)-water (50%-50%) propolis extract was administered (100 mg/kg).
- Group 7 (DMSO): DMSO water (50%-50%) was administered as a negative control (equal volume DMSOEP).
- Group 8 (OOEP): Olive oil (cold-pressed Memecik olive oil from Milas) propolis extract was administered (100 mg/kg).
- Group 9 (OO): Olive oil was administered as a negative control (equal volume OOEP).

The volume of solvents (W, EW, DMSO, OO) used for the negative control was the same as that of propolis extracts given for the experiment. Mice were euthanised by cervical dislocation under anaesthesia with 10 mg/kg xylazine hydrochloride (Rompun®, Bayer, 23.32 mg/ml, Germany) followed by 70 mg/kg ketamine hydrochloride (Ketalar®, Parke-Davis, 50 mg/ml, Germany) one hour after indomethacin administration, and their stomachs were removed and sent for macroscopic histopathological examination in 10% formaldehyde (de Barros et al., 2007; Ruiz-Hurtado et al., 2021b)

Histopathologic Analysis

Stomach samples were fixed in 10% formaldehyde solution. After fixation, the tissues were processed through an alcohol and xylol series and embedded in paraffin blocks. Sections of 3-5 µm thickness were transferred from the paraffin blocks to microscope slides, stained with haematoxylin-eosin, and then examined microscopically. In the histopathological examination, the groups were scored semiquantitatively, with slight modifications, for (1) epithelial cell loss (score: 0-3), (2) hemorrhage (score: 0-3), (3) inflammatory cell infiltration (score: 0-3), (4) lamina propria mucosal erosions (score: 0-3), (5) edema (score: 0-3). The scoring was determined as follows: 0: none, 1: light, 2: medium, 3: violent (Yang et al., 2017).

Statistical Analysis

Statistical analyses of the results were performed with the software Minitab 21.0.1. The Mann-Whitney U test was used to compare the means of the each groups. The assessment of significance levels was evaluated according to $P \leq 0.05$.

Results

The pathological results of the study were evaluated as a positive control of omeprazole, and the solvent of each propolis application was a negative control. Scores of mean histopathological results are given in Table 1 and Figure 1.

Pathological examination revealed that epithelial loss was lowest in the OMP group and highest in the EEP group among the treatment groups (Table 1 and Figure 1).

Mild epithelial loss was observed in the groups. It was seen that the OMP group was the most successful in treatment with less epithelial loss, followed by the DMSO group with more homogeneous results. With regard to bleeding, there were significant findings in the alcohol groups, whereas no bleeding was observed in the WEP group. In the OOEP, DMSOEP and OMP groups, bleeding was observed in only one animal, indicating that these groups also successfully prevented bleeding after the WEP group. The EEP group had mild to moderate inflammatory cell infiltration, whereas mild infiltration was observed in the other groups (Table 1 and Figure 2-3).

Similarly, erosion and edema were more prominent in the EEP group than in the others. When all the results were evaluated, it was determined that the scores closest to the treatment group (OMP) were found in the DMSOEP and OOEP groups, respectively. In the negative control groups (W, EW, OO, DMSO), the most prominent findings regarding all histopathological changes were observed in the EW group. In contrast, the conclusions of the OO and DMSO groups were observed to be attenuated (although not as therapeutically). It was also determined that epithelial loss in the EW group was violent, bleeding persisted, the inflammatory response continued significantly, although not very severely, and edema, particularly, was found to be violent (Table 1 and Figure 1).

In the statistical analysis of the pathological results with the mean clinical scores, it was found that the results closest to the OMP treatment group were DMSOEP and OOEP, and there was no statistical difference between them. The other treatments were found to have a negative significant difference compared to OMP, which means that they had no therapeutic effect. Both clinical scoring and pathological examination results show that DMSOEP and OOEP treatments have gastroprotective efficacy in indomethacin-induced gastric ulcers. In addition, EWEP treatment has been found to have adverse effects on epithelial loss, erosion, edema formation and bleeding (Table 1 and Figure 1).

Discussion

Gastric ulcer is a significant health problem that causes gastrointestinal complications such as bleeding and perforation in both humans and animals caused by various drugs, chemicals and stress (Ruiz-Hurtado et al., 2021a). Therefore, many studies have been conducted to determine the gastroprotective effect (De Barros et al., 2008; Ruiz-Hurtado et al., 2021a). In gastroprotective studies, the efficacy of NSAID drugs such as indomethacin or acetic acid was evaluated by using many natural agents such as propolis before ulcer formation (Liu et al., 2002; Mohafez et al., 2010; Pillai et al., 2010; El-Ghazaly et al., 2011; Abd El-Hady et al., 2013; Costa et al., 2020; de Mendonça et al., 2020; Badriyya et al., 2021; Boeing et

Table 1. Pathological results of gastric tissue [Mean (SD)]3

Treatment groups2	Mean clinical scores	Epithelial loss	Bleeding	Inflammatory cell infiltration	Erosion	Edema
OMP (+)	2.13 (0.84)	0.50 (0.54)	0.13 (0.35)	0.88 (0.35)	0.00 (0.00)	0.75 (0.46)
WEP	3.38 (1.06)	1.13 (0.35)	0.00 (0.00)	1.00 (0.00)	0.00 (0.00)	1.25 (0.89)
W(-)	5.13 (3.14)	1.25 (0.46)	0.63 (1.06)	1.63 (0.74)	0.50 (0.76)	1.13 (0.64)
EEP	7.13 (2.70)	1.63 (0.74)	1.25 (0.89)	1.63 (0.52)	1.25 (0.71)	1.50 (0.93)
WE (-)	9.38 (2.45)	2.13 (0.64)	1.63 (1.19)	1.88 (0.35)	1.13 (0.64)	2.38 (0.74)
DMSOEP	2.75 (1.04)	1.00 (0.54)	0.13 (0.35)	1.00 (0.00)	0.00 (0.00)	0.63 (0.52)
DMSO (-)	3.25 (2.25)	0.88 (0.84)	0.38 (0.52)	0.88 (0.35)	0.13 (0.35)	1.00 (0.76)
OOEP	2.88 (1.36)	0.88 (0.84)	0.13 (0.35)	0.88 (0.35)	0.25 (0.46)	0.88 (0.35)
OO (-)	4.38 (2.26)	1.25 (0.71)	0.38 (0.52)	1.00 (0.54)	0.50 (0.76)	1.25 (0.71)
P values1						
WEP	0.020	0.015	0.334	0.312	1.000	0.180
W(-)	0.010	0.010	0.227	0.022	0.008	0.201
EEP	0.010	0.010	0.005	0.004	0.002	0.060
WE (-)	0.010	0.004	0.004	0.002	0.001	0.001
DMSOEP	0.205	0.080	0.997	0.335	1.000	0.619
DMSO (-)	0.030	0.030	0.278	0.028	0.564	0.438
OOEP	0.199	0.030	0.998	0.798	0.146	0.554
OO (-)	0.020	0.031	0.279	0.590	0.040	0.116

[1]Statistical comparison of positive control omeprazole with other treatments.
[2]Omeprazole: OMP, water extract propolis: WEP, only water: W, Hydro-alcoholic extract propolis: EEP, only water-ethanol: WE, dimethyl sulphoxide extract propolis: DMSOEP, only dimethyl sulphoxide: DMSO, olive oil extract propolis: OOEP, only olive oil: OO, (+): positive treatment control, (-): negative treatment control, Standard deviation: SD, Pooled Standard deviation: P-SD
[3]n = 8 per treatment group

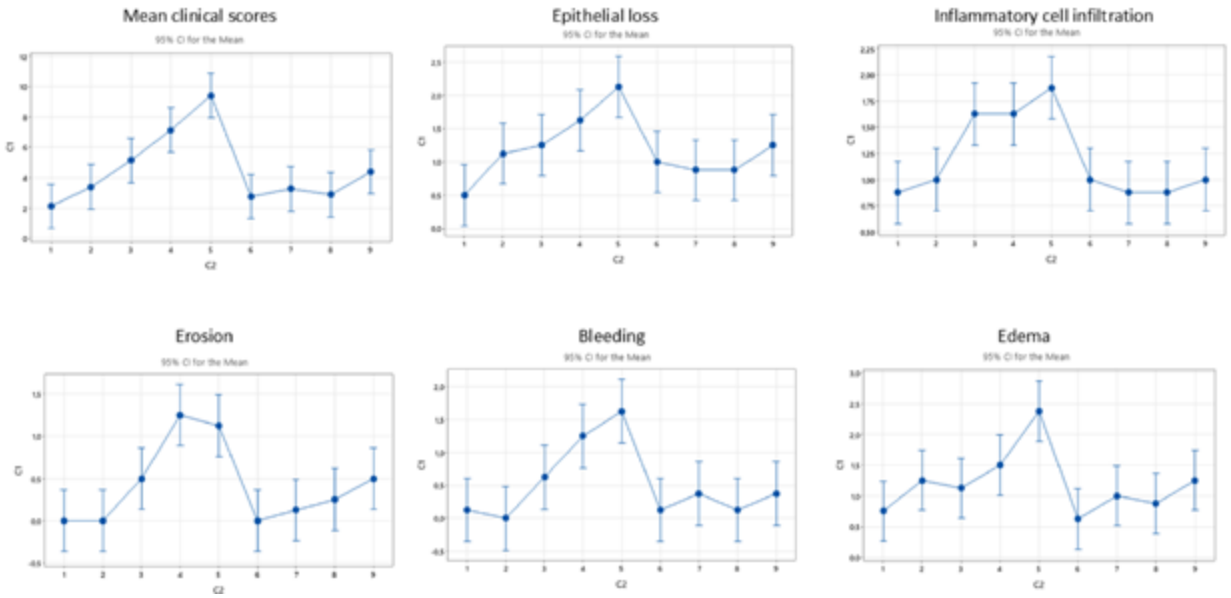

Figure 1. Means of pathology scoring results of the gastric tissue.

The pooled standard deviation is used to calculate the intervals. C1 is mean of pathological scores ± SD, C2 is treatment groups. Omeprazole: OMP, water extract propolis: WEP, only water: W, Hydro-alcoholic extract propolis: EEP, only water-ethanol: WE, dimethyl sulphoxide extract propolis: DMSOEP, only dimethyl sulphoxide: DMSO, olive oil extract propolis: OOEP, only olive oil: OO, (+): positive treatment control, (-): negative treatment control.

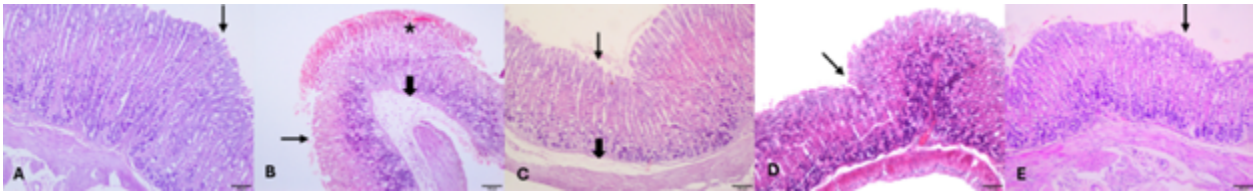

Figure 2. The histopathology of the treatments of propolis extracts and omeprazole.

A: Olive oil extract propolis, B: Hydro-alcoholic extract propolis, C: Water extract propolis, D: Dimethyl sulphoxide extract propolis E: Omeprazole, Arrow; epithelium (mucosa), Star; bleeding areas, Bold arrow; edema and cell infiltration (H&E staining, 20x).

al., 2021; Ruiz-Hurtado et al., 2021b; Boeing et al., 2023; Oyetayo et al., 2023; Sahin et al., 2023). The evaluations are compared with the solvent of the extract used with gastroprotective drugs such as proton pump inhibitor or H2 receptor antagonist, etc. and their gastroprotective activities are studied. (Liu et al., 2002; Pillai et al., 2010; Mohafez et al., 2010; El-Ghazaly et al., 2011; Abd El-Hady et al., (2010) Indian propolis similar to cimetidine. In the current study, no difference was found between propolis extracted with 70% ethanol, as in other studies, and the solvent ethanol only, except for preventing edema formation. In addition, the lowest gastroprotective effect of the four extracts was observed in the EEP when compared to omeprazole, the positive control treatment.

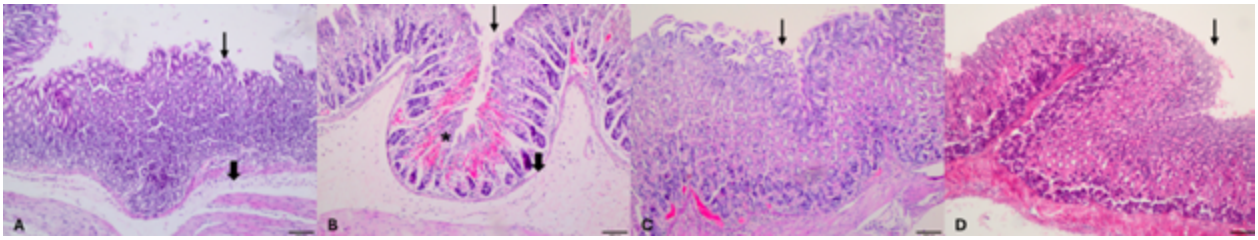

Figure 3. The histopathology of the negative controls.

A: Only olive oil, B: Only water-ethanol, C: Water, D: Only dimethyl sulphoxide, Arrow; epithelium (mucosa), Star; bleeding areas, Bold arrow; edema and cell infiltration (H&E staining, 20x).

et al., 2013; Costa et al., 2020; de Mendonça et al., 2020; Badriyya et al., 2021; Boeing et al., 2021; Ruiz-Hurtado et al., 2021b;Boeing et al., 2023; Oyetayo et al., 2023; Sahin et al., 2023). In the present study, similar to previous studies, the gastroprotective activity of four different propolis extracts was evaluated in mice using the same model. Previous studies have examined the efficacy of propolis extracted with 70% ethyl alcohol and water or propolis lyophilised after extraction with alcohol and then dissolved in water (Pillai et al., 2010; El-Ghazaly et al., 2011; Boeing et al., 2021; Sahin et al., 2023). Another study examined the efficacy of propolis extracted with acetone by dissolving in water after lyophilisation (Oyetayo et al., 2023). In all previous studies, a significant gastroprotective activity of ethanol-extracted propolis was observed (Liu et al., 2002; Mohafez et al., 2010; Pillai et al., 2010;Abd El-Hady et al., 2013; Costa et al., 2020; de Mendonça et al., 2020; Boeing et al., 2021; Ruiz-Hurtado et al., 2021b; Boeing et al., 2023; Sahin et al., 2023). Studies investigating the gastroprotective activity of propolis extracted with ethyl alcohol have shown remarkable similarities with conventional antiulcer drugs, which are Boeing et al., (2023) Brazilian red propolis, de-Mendonça et al., (2020) Brazilian red propolis, Oyetayo et al., (2023) Nigerian propolis and Ruiz-Hurtado et al., (2021b) Mexican propolis similar to omeprazole, Boeing et al., (2021) Brazilian red propolis similar to carbenoxolone, Costa et al., (2021) Brazilian green propolis similar to omeprazole, ranitidine and carbenoxolone, Abd-El Hady et al., (2013) Egyptian propolis similar to ranitidine, Mocam et al., (2024) Cameroonian propolis similar to sucralfate, Pillai Similarly, the least gastroprotective effect was observed in the EEP in the total clinical score examination, and no difference was found with the group that was administered 70% ethanol only. Many studies claim that the gastroprotective effect of propolis is due to the phenolic compounds it contains (Costa et al., 2020; de Mendonça et al., 2020; Ruiz-Hurtado et al., 2021b). Although previous studies have shown that the highest phenolic compounds were also obtained in alcohol extract propolis, the least effect was determined in EEP in present study (Kekeçoğlu and Sorucu, 2021). Badriyya et al. (2021) non-alcoholic commercial propolis was used in a study in mice against aspirin-induced gastric ulcers and showed a significant protective effect. While most studies have been carried out in rats, the present study was conducted in mice, similar to the study by Badriya et al., (2021). In addition, El-Ghazly et al., (2011) determined that water extract of propolis applied to rats against indomethacin-induced ulcers had as much protective activity as lansoprazole. In the present study, the gastroprotective activity of the water extract propolis was slight but not as strong as omeprazole. There was no statistical difference between WEP and W in terms of gastroprotection in preventing bleeding and edema. On the other hand, gastroprotective activity was observed, and there was a statistically significant difference between WEP and W in the total clinical score evaluation. Şahin et al. (2023) determined that water and ethanol extract propolis had gastroprotective effects and water extract propolis was more effective, which was the only study compared with different solvents. The fact that the water extract was

better than the ethanol extract corroborates our results. In the current study, DMSOEP and OOEP were found to be the best solvents in terms of gastroprotective effects on the stomach. In the statistical analysis, although some parameters were not found to be different with solvents when histopathological evaluations were performed separately, a significant statistical difference occurred in the overall clinical score evaluation. Although DMSO, one of these solvents, is not widely used due to some toxic effects, propolis extracted in olive oil is essential for safe use in terms of stomach protection.

Conclusion

The present study investigated and compared the gastroprotective activity of propolis extracted with various solvents. The results showed that the water extract of propolis showed a slight effect, and the ethanol extract did not. Although the gastroprotective activity of propolis is due to its phenolic compounds, the ineffectiveness of the ethanolic extract in which these substances are highly concentrated is a situation that needs to be clarified. This situation can be explained by the fact that alcohol also accelerates ulcer formation and even creates an ulcer model. However, the reason why the phenolic compounds it contains are ineffective here should be investigated. DMSO and olive oil extracts were found to be more effective than other solvents. In addition, both propolis extracts were found to have as much of a gastroprotective effect as omeprazole. In addition, the compounds in DMSO and olive oil extracts that have this effect need to be studied in the future. In conclusion, although DMSO, one of these solvents, is not widely used due to some toxic effects, propolis extracted in olive oil is important for safe use in terms of stomach protection.

Acknowledgements

The study supported by TUBİTAK 1919B012109713 project number (TUBİTAK 2209 A)

Author contribution statement

Concept and design: A.S, O.B., A.A., A.T; Data collection or processing: A.S., A.A.; Analysis or interpretation: A.A.; Literature search: A.A, O.B.; Writing: A.A, O.B, A.T.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Abd El-Hady, F.K., El Awdan, S.A., & Ibrahim, A.M. (2013). Anti-ulcerative potential of Egyptian propolis against oxidative gastric injury induced by indomethacin in rats. *Asian J Med Pharm Res,* 3, 35-42.

Abumunaser, A. (2021). Peptic Ulcer Disease. In *Cases on Medical Nutrition Therapy for Gastrointestinal Disorders* (pp. 46-67): IGI Global. https://doi.org/10.4018/978-1-7998-3802-9.ch003

Badriyya, E., Mandasari, Z., Aldi, Y., & Almahdy, A. (2021). Gastroprotective Effect of Propolis Against Male White Mice Gastric Ulcers Induced by Aspirin. *Paper presented at the 2nd International Conference on Contemporary Science and Clinical Pharmacy* (ICCSCP 2021). https://doi.org/10.2991/ahsr.k.211105.014.

Boeing, T., Mejia, J. A.A., Ccana-Ccapatinta, G.V., Mariott, M., de Souza, P., Mariano, L. N.B., & de Andrade, S.F. (2021). The gastroprotective effect of red propolis extract from Northeastern Brazil and the role of its isolated compounds. *Journal of Ethnopharmacology,* 267, 113623. https://doi.org/10.1016/j.jep.2020.113623

Boeing, T., Monteiro Magalhaes de Oliveira, B., Aldana-Mejía, J.A., Vidal Ccana-Ccapatinta, G., Venzon, L., Judah Cury, B., &Mota da Silva, L. (2023). Brazilian Red Propolis Accelerates Gastric Healing and Reduces Gastric Submucosal Layer Inflammation in Ultrasound-Monitored Rats. *Chemistry & Biodiversity,* 20(1), e202200992. https://doi.org/10.1002/cbdv.202200992

Costa, P., Somensi, L.B., da Silva, R.d.C.M.V.d.A.F., Mariano, L.N.B., Boeing, T., Longo, B., & Pellizzon, C.H. (2020). Role of the antioxidant properties in the gastroprotective and gastric healing activity promoted by Brazilian green propolis and the healing efficacy of Artepillin C. *Inflammopharmacology,* 28, 1009-1025. https://doi.org/10.1016/j.jep.2020.113623

De Barros, M.P., Lemos, M., Maistro, E.L., Leite, M.F., Sousa, J.P.B., Bastos, J.K., & de Andrade, S.F. (2008). Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. *Journal of Ethnopharmacology,* 120(3), 372-377. https://doi.org/10.1016/j.jep.2008.09.015

de Barros, M.P., Sousa, J.P.B., Bastos, J.K., & de Andrade, S.F. (2007). Effect of Brazilian green propolis on experimental gastric ulcers in rats. *Journal of Ethnopharmacology,* 110(3), 567-571. https://doi.org/10.1016/j.jep.2006.10.022

de Mendonça, M.A., Ribeiro, A.R., Lima, A.K.d., Bezerra,G.B., Pinheiro, M.S., de Albuquerque-Júnior, R.L., & Novellino, E. (2020). Red propolis and its dyslipidemic regulator formononetin: Evaluation of antioxidant activity and gastroprotective effects in rat model of gastric ulcer. Nutrients 12*(10*), 2951. https://doi.org/10.3390/nu12102951

Ekeuku, S.O., & Chin, K.Y. (2021). Application of propolis in protecting skeletal and periodontal health-A systematic review. *Molecules,* 26(11), 3156. https://doi.org/10.3390/molecules26113156.

El-Ghazaly, M.A., Rashed, R.R.A., & Khayyal, M.T. (2011). Anti-ulcerogenic effect of aqueous propolis extract and the influence of radiation exposure. *International Journal of Radiation Biology,* 87(10), 1045-1051. https://doi.org/10.3109/09553002.2011.602378

Kekeçoğlu, M., & Sorucu, A. (2021). Determination of The effect of green extraction solvents on the phenolic acids and flavonoids of propolis. *Journal of Research in Veterinary Medicine,* 41(1), 49-54. https://doi.org/10.30782/jrvm.937418

Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A., & Smolic, M. (2019). Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. *Journal of Clinical Medicine*, 8(2), 179. https://doi.org/10.3390/jcm8020179

Liu, C.F., Lin, C.C., Lin, M.H., Lin, Y.S., & Lin, S.C. (2002). Cytoprotection by propolis ethanol extract of acute absolute ethanol-induced gastric mucosal lesions. *The American Journal of Chinese Medicine,* 30(02n03), 245-254. https://doi.org/10.1142/S0192415X02000387

Malfertheiner, P., Chan, F.K., & McColl, K.E. (2009). Peptic ulcer disease. *The Lancet,* 374(9699), 1449-1461. https://doi.org/10.1016/S0140-6736(09)60938-7

Mohafez, O.M.M., Abdel-Raheem, I. T., & Nafady, A.M. (2010). Antioxidant, Lipid Peroxidation-Inhibitory and Antiulcer Activities of Brown Propolis. *Bulletin of Pharmaceutical Sciences*, 33, 169-177. https://doi.org/10.21608/BFSA.2010.64741

Oruç, H.H., Çayci, M., Sorucu, A., Uzabacı, E., & Nyandwi, R. (2023). Characterization of commercially available propolis products in Turkey based on individual phenolic compounds. *Journal of Apicultural Research,* 62(5), 1225-1232. https://doi.org/10.1080/00218839.2021.1962110

Oyetayo, N.S., Kodie, D.O., Nwakasi, M.I., Afolabi, O.O., Jarikre, T.A., Eyarefe, O.D., & Emikpe, B.O. (2023). Gastroprotective and ulcer healing potentials of Nigerian Bee Propolis flavonoid extract on acetic acid-induced gastric ulcers in albino rats (Wistar Strains). *Advances in Traditional Medicine,* 23(4), 1221-1230. https://doi.org/10.1007/s13596-022-00674-y

Pillai, S. I., Kandaswamy, M., & Subramanian, S. (2010). Antiulcerogenic and ulcer healing effects of Indian propolis in experimental rat ulcer models. *Journal of ApiProduct and ApiMedical Science,* 2(1), 21-28. https://doi.org/10.3896/IBRA.4.02.1.02

Ramakrishnan, K., & Salinas, R.C. (2007). Peptic ulcer disease. *American Family Physician, 76*(7), 1005-1012.

Ruiz-Hurtado, P.A., Garduño-Siciliano, L., Domínguez-Verano, P., Balderas-Cordero, D., Gorgua-Jiménez, G., Canales-Alvarez, O., & Rodríguez-Monroy, M.A. (2021a). Propolis and Its Gastroprotective Effects on NSAID-Induced Gastric Ulcer Disease: A Systematic Review. *Nutrients,* 13(9). https://doi.org/10.3390/nu13093169

Ruiz-Hurtado, P.A., Garduño-Siciliano, L., Dominguez-Verano, P., Martinez-Galero, E., Canales-Martinez, M.M., & Rodriguez-Monroy, M.A. (2021b). Evaluation of the gastroprotective effects of Chihuahua propolis on indomethacin-induced gastric ulcers in mouse. *Biomedicine & Pharmacotherapy,* 137, 111345. https://doi.org/10.1016/j.biopha.2021.111345.

Sahin, H., Kaltalioglu, K., Erisgin, Z., Coskun-Cevher, S., & Kolayli, S. (2023). Comparison of Protective Effect of Aqueous and Ethanolic Extracts of Propolis against Gastric Ulceration in Rats. *Chemistry & Biodiversity,* 20(5), e202300305. https://doi.org/10.1002/cbdv.20230030

Sorucu, A. (2019). Arı Ürünleri ve Apiterapi. *Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni,* 10(1), 1-15. ISSN: 1309-4769

Sorucu, A., & Oruç, H.H. (2019). Determination of biologically active phenolic compounds in propolis by LC–MS/MS according to seasons and altitudes. *Journal of Food Measurement and Characterization,* 13, 2461-2469. https://doi.org/10.1007/s11694-019-00166-9

Stojanović, S. T., Najman, S.J., Popov, B.B., & Najman, S.S. (2020). Propolis: chemical composition, biological and pharmacological activity–a review. *Acta Medica Medianae,* 59(2). https://doi.org/10.5633/amm.2020.0215

Yang, Y., Yin, B., Lv, L., Wang, Z., He, J., Chen, Z., Li, Y. (2017). Gastroprotective effect of aucubin against ethanol-induced gastric mucosal injury in mice. *Life Sciences,* 189, 44-51. https://doi.org/10.1016/j.lfs.2017.09.016